THE ACTIVE SITE OF GLUCOSE PHOSPHATE ISOMERASE

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1. Introduction

D-Glucose 6-phosphate ketol isomerase (EC 5.3.1.9) has been the subject of recent investigation in several laboratories, and the structure to high resolution of its companion isomerase in the glycolytic sequence, triose phosphate isomerase, has been published in the past year [1]. A structural basis for proposals about the mechanism of action of isomerases is therefore beginning to emerge. As a preliminary contribution, we give some aspects of the results of crystallographic work on glucose phosphate isomerase isolated from pig skeletal muscle.

2. Results

The solution of the crystal structure of this protein to a resolution of 6 Å has been reported [2], together with a tentative identification of the substrate binding site using the inhibitor gluconate 6-phosphate. Chemical studies have shown the enzyme to be a dimer, with an approx. mol. wt. of 120 000, and in the crystal the subunits of the dimer are related by a crystallographic dyad axis [2]. At 6 Å resolution the molecule appeared approximately spherical and it was possible to delineate the boundary between the two subunits. The inhibitor was bound to a site very close to this boundary and there were associated conformational changes in this region of the enzyme.

The resolution of this structure has now been extended to 3.5 Å; phase angles were estimated by isomorphous replacement using two heavy atom derivatives with anomalous scattering measurements on each derivative. (The mean figure of merit over all independent reflections (~8000) was 0.64). The course of the polypeptide chain has been traced and a molecular model of the structure has been built; the electron density belonging to one subunit was fitted by a chain of 490 residues. As amino acid sequence data was not available the chain was built as polyglycine, but side-group density was visible for the majority of residues.

Interpretation of the map was made easier by the large amount of secondary structure present. Each subunit contains two β-sheets – one interpreted as four parallel strands, the other as six parallel strands – and the strands of β-sheet are interconnected by α-helices. In the smaller sheet each β strand is laid down next to the previous one, but in the larger sheet the interconnections do not follow a simple pattern. The subunits are closely associated; the innermost strand of the larger β-sheet is close to and collinear with the molecular dyad axis, and this sheet is therefore continuous with its symmetrically related counterpart.

Crystallization of the enzyme in the presence of the powerful competitive inhibitor arabinonate 5-phosphate ($K_i = 3 \times 10^{-7}$ M below pH 7 [3]) gave crystals that were isomorphous with the unmodified form, and a difference Fourier map between the native enzyme and this complex was, at 6 Å, very similar indeed to the gluconate 6-phosphate difference map. By extending to 3.5 Å the resolution of data on the arabinonate phosphate complex it has proved possible to fix the probable conformation of the inhibitor, to delineate the regions of the enzyme that must interact with it, and to locate in the electron

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density map of the native enzyme many of the groups that must be concerned with the interaction.

The binding site is a fairly well enclosed pocket, formed partly in the slight cleft between the two β-sheet domains of one subunit, and partly by portions of chain from the other subunit. On one side of the pocket, the phosphate group of the inhibitor is bound by a loop of chain at the C-terminal end of the smaller β-sheet (see fig.1). Another portion of chain from the same subunit forms the lower part of this side of the pocket. On the other side of the pocket part of the chain from the adjoining subunit has moved towards the bound inhibitor, bringing two large side-groups (D and E in fig.1) into a position

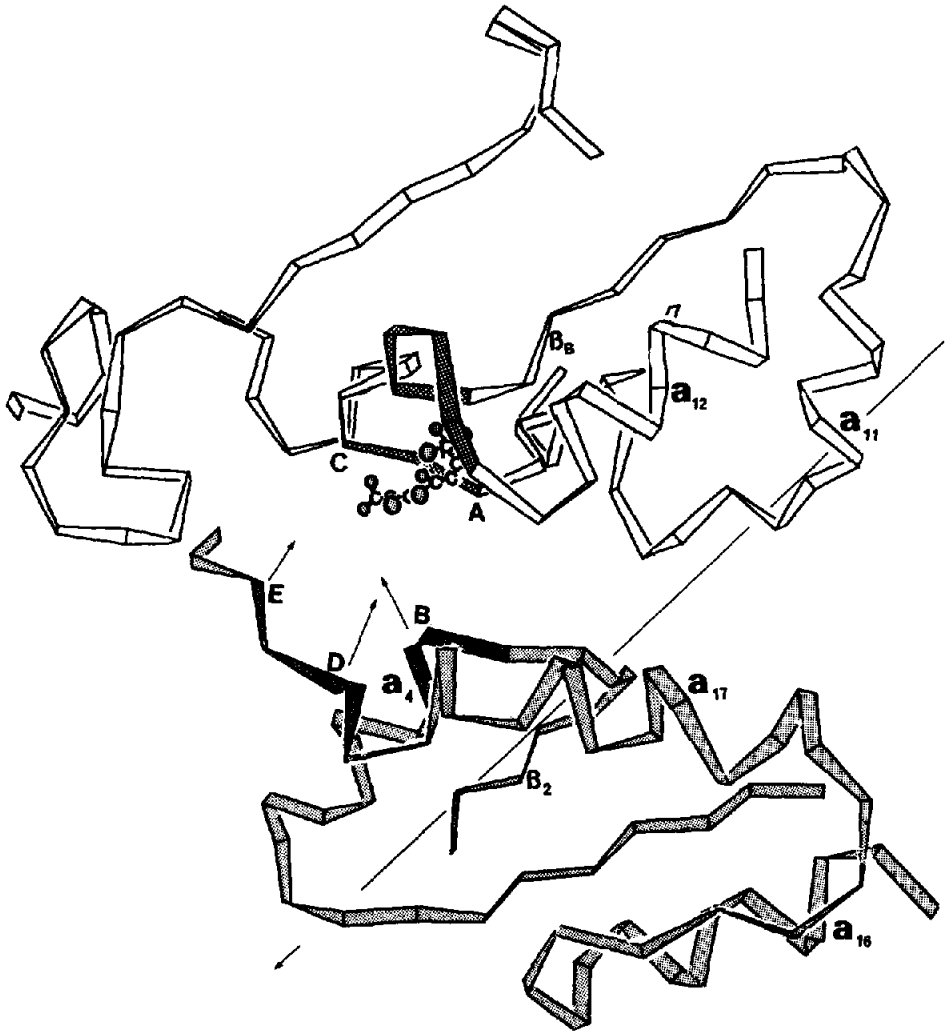

Fig.1. The folding of the polypeptide chain around the active site of glucose phosphate isomerase. The inhibitor, arabinonate 5-phosphate is shown in the site. The chain is shown in its conformation in the native enzyme; the movements of protein that accompany the binding of the inhibitor are indicated by arrows. The amino acid side chains implicated in binding and catalysis are labelled A to E, and the portions of chain forming the active site pocket are indicated by dark shading. The chain in one subunit has been lightly shaded, that in the other left blank. Secondary structural elements have been labelled α (α-helices) and β (parallel β-sheet). The labelling scheme adopted for the subunit was $\beta_1\beta_8$ for the larger β-sheet and β_A–β_D for the smaller. The molecular dyad axis has been shown by a double-ended arrow.

close enough to interact with OH_3 and OH_2 respectively of the inhibitor. Another large group (B in fig.1) has moved towards C_1 of the bound molecule.

3. Discussion

Investigators who have studied the mechanism of isomerases, notably Rose and O'Connell [4], have reached the conclusion that the isomerisation reaction involves the transfer of a proton between C_1 and C_2 of the substrate, with the formation of an intermediate ene-diol, whose configuration must be cis- for all the enzymes that have been studied. In the case of glucose phosphate isomerase the base mediating proton transfer cannot contain exchangeable hydrogen atoms [4] and Dyson and Noltmann have suggested imidazole as the most likely candidate [5]. It has been shown [6] that the preferred substrates are the α-anomers of D-glucose 6-phosphate pyranose and D-fructose 6-phosphate furanose (although the β-anomers are probably isomerised at a much slower rate). By far the most abundant form of the glucose substrate in solution is the Cl conformer (chair with substituents on C_2 to C_5 equatorial) [7], and the crystallographic results have shown that the enzyme-bound conformation is probably close to this. The enzyme must, therefore, catalyse opening of the sugar ring, and this could be accomplished either as a separate step prior to, or in a concerted manner synchronous with, enolisation; the consecutive pathway seems the more likely in view of the fact that this enzyme catalyses the anomerisation of mannose 6-phosphate [8].

It has also been shown [7] that there is hydrogen-bond donation to the enzyme from OH_3 and OH_4 of the substrate; we should, therefore, expect groups D and E in fig.1 to be capable of hydrogen-bond acceptance. Interaction from the protein with OH_1 and OH_2 of the substrate seems likely, both to give the great substrate specificity and to promote transition to the geometry of the cis-ene-diol intermediate. Examination of the electron density map of the native enzyme shows that there is an amino acid side-group (C in fig.1) which could easily interact with OH_1 or OH_2 of the substrate. O'Connell and Rose [9] have labelled a glutamate residue by esterification at C_1 of the epoxide 1,2-anhydromannitol 6-P. In explanation, it was suggested that if considerable torsion about C_2–C_3 of the bound inhibitor could take place the epoxide ring could be brought into the correct geometry for attack by the group that mediates proton transfer in the isomerisation. However, the group we have implicated in the interaction at OH_1 and OH_2 of the substrate would, without the necessity for any torsion of the inhibitor, be very close to the required geometry for this esterification, and we suggest that this may be the labelled glutamate residue. It is proposed that such a carboxylate group could form hydrogen-bonds with both OH_1 and OH_2 of the substrate, possibly promoting torsion of C_1–C_2 to bring OH_1 and OH_2 into an eclipsed relation (see fig.2: 1 and 2).

Dyson and Noltmann have also proposed general acid catalysis of ring-opening by lysyl ammonium [5]; while agreeing that acid catalysis is likely, we prefer a concerted acid/base catalysis, with the proton transfers a natural extension of the binding interactions. This, we propose, may be followed by another acid/base catalysis of enolisation (see fig.2).

The ring-opening step constitutes a charge relay process; a positive charge (originating on group A) is transferred from a region which may be expected to be shielded by the substrate from the external solvent, to the carboxylate group C and then to the base B, both of which are near the surface of the molecule and probably more accessible to water. It will be interesting to discover whether this relay of charge contributes to the catalysis.

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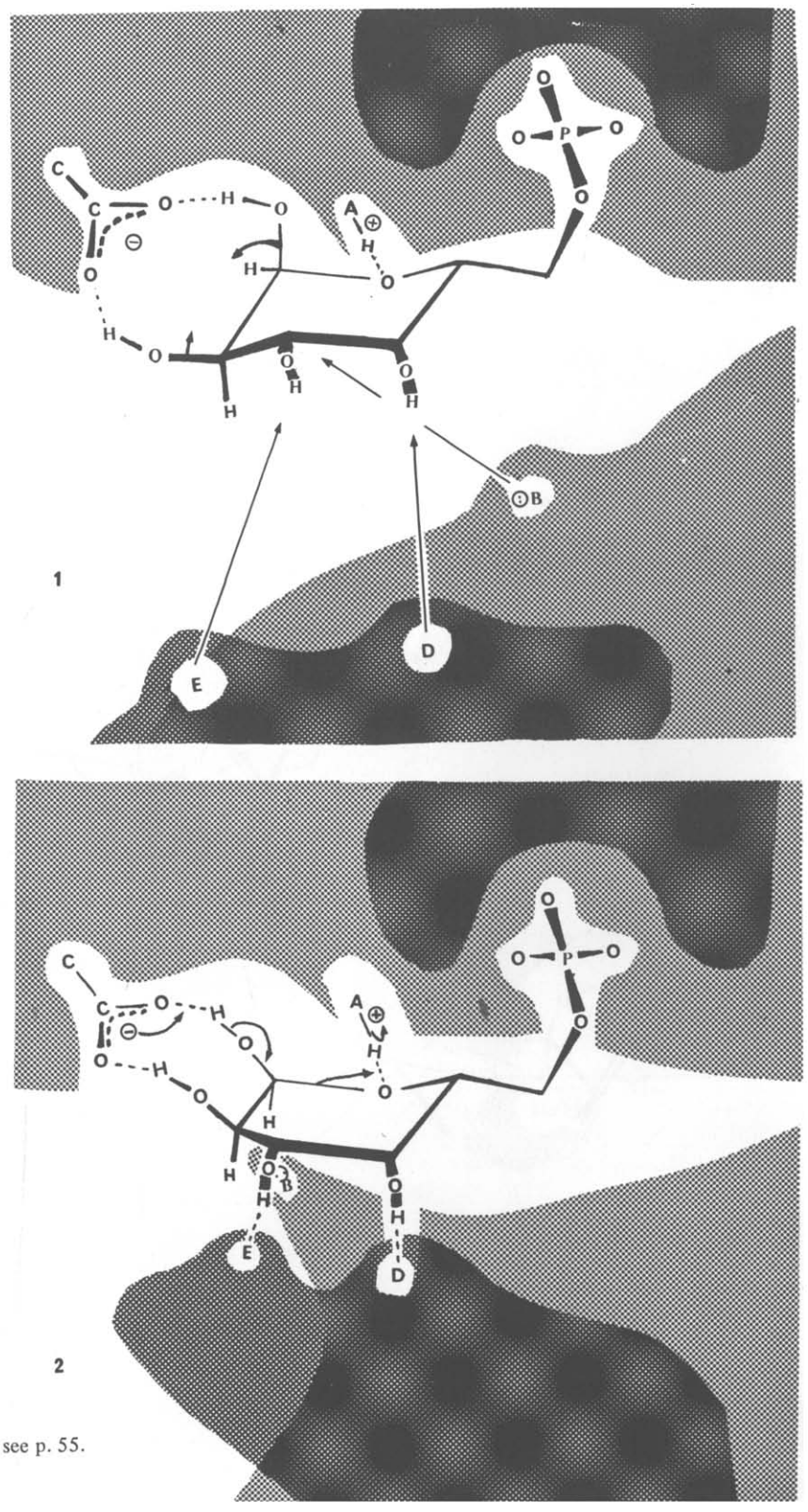

Fig.2. For caption see p. 55.

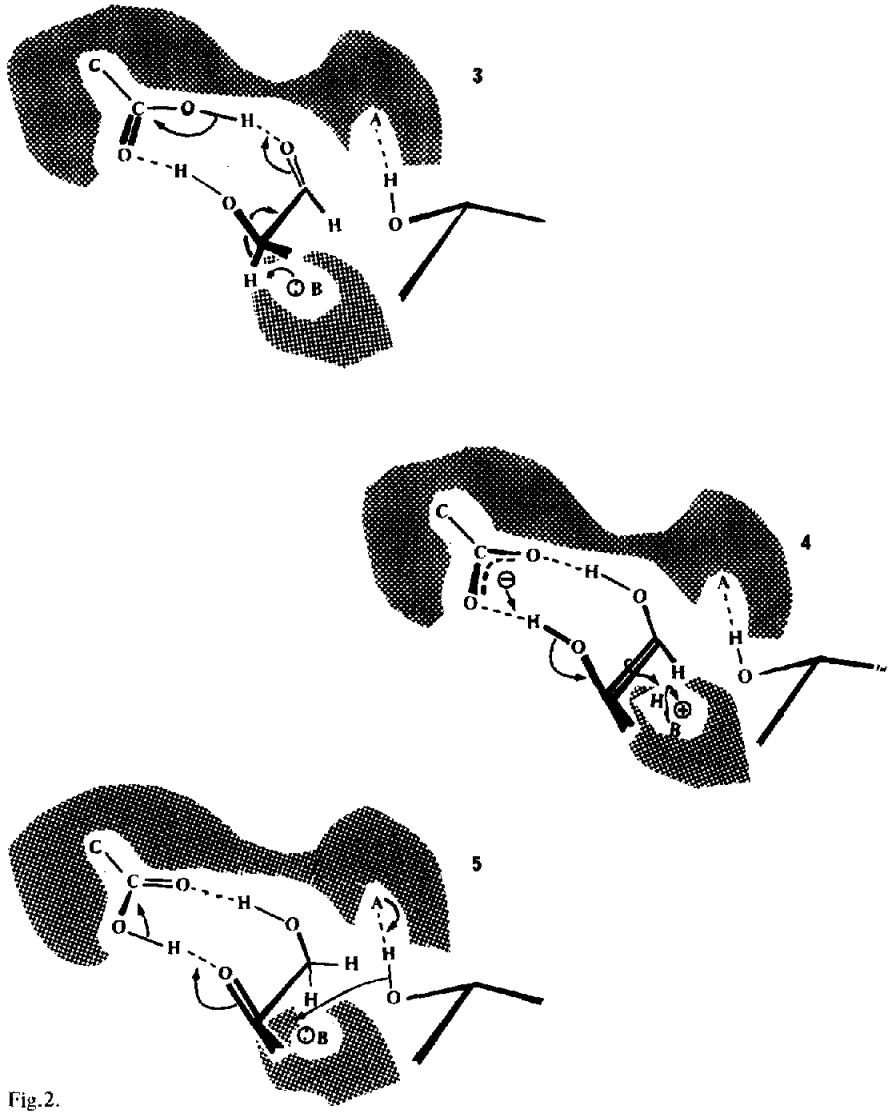

Fig.2.

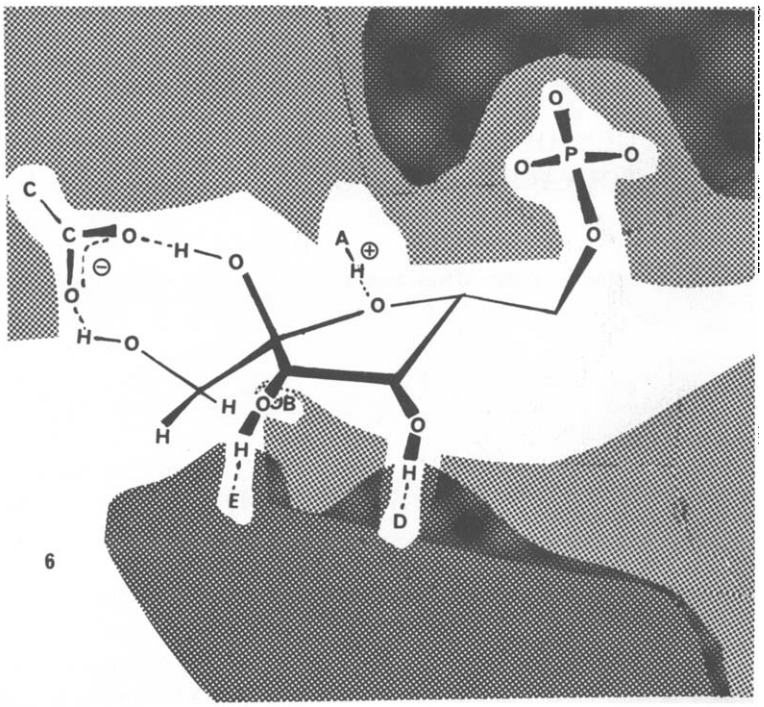

Fig.2. Schematic diagram of a proposal for some aspects of the mechanism of action of glucose phosphate isomerase. The shaded areas represent the polypeptide chain around the active site. 1 and 2 show a stylised diagram of the conformational changes taking place in the protein (c.f. fig.1) and those that may take place in the substrate. For convenience the movements of protein have been drawn as taking place after the binding of the substrate, but it is impossible to say from these studies whether they take place before or after binding. It is also impossible to tell whether the protein movements occur during each reaction cycle, or represent the transition of the enzyme to a different conformational state in the presence of substrate. We have suggested that a torsion of C_1-C_2 may occur before ring-opening. If this is the case the induction of strain into the substrate and its subsequent removal on ring-opening could be a contributory factor in the enzymatic catalysis. The labels A to E represent the same side-groups as in fig.1. The ionisation state of the phosphate group of the substrate has not been indicated.

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